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COMMUNICATION

UV Inactivation of *E. coli* in Liquid Egg White

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Abstract Liquid egg white is currently pasteurized using heat; however, this treatment damages the functional properties of the egg. In this study, a nonthermal ultraviolet light (UV) system was developed to pasteurize liquid egg white. The system consisted of low-pressure mercury bulbs surrounded by UV transparent tubing. Egg white was inoculated with *Escherichia coli* K12 and pumped through the UV system at a flow rate of 330 ml/min. The effects of treatment time (0 to 160 s), temperature (30 to 50 °C), and egg white pH (7 to 9) on the inactivation of *E. coli* were investigated. The population of *E. coli* in egg white was reduced by 4.3 log after being exposed to UV at 50 °C for 160 s. Inactivation was linearly dependent on treatment time and was adequately described using first-order kinetics (r^2 of 0.94). The electrical energy of the process was calculated to be 44 J/ml. Inactivation was directly dependent on temperature and inversely dependent on pH. Nonthermal UV processing has the potential to improve the safety and functional properties of liquid egg white.

Keywords Nonthermal · Pasteurization · Egg white · *E. coli* · Ultraviolet light

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Introduction

The thermal pasteurization of liquid egg white is problematic because of its great instability in the range of effective pasteurization temperatures (Stadelman and Cotterill 1995). Therefore, nonthermal processes to pasteurize liquid egg whites have been investigated. These include pulsed electric fields (Amiali et al. 2006; Fernandez-Diaz et al. 2000; Jeantet et al. 1999), electron beam irradiation (Wong et al. 1996), as well as gamma irradiation and high hydrostatic pressure (Andrassy et al. 2006). Similar to thermal pasteurization, nonthermal pasteurization of egg white is challenging. Pulsed electric field processing of egg whites has only occurred on a small scale to date; the maximum flow rate is 6 ml/min (Amiali et al. 2006). The term "irradiation" is easily misunderstood by consumers and special interest groups vocally oppose the process (Bruhn 2005). High hydrostatic pressure processing of egg whites can cause protein denaturation, aggregation, and coagulation (Andrassy et al. 2006). Despite substantial efforts, none of the nonthermal processes have yet to be commercialized for egg whites.

Another potential nonthermal process for pasteurization of egg white is ultraviolet light (UV). Food processors have made use of UV for decades. The 1943 edition of the Carnation Cook Book stated that Irradiated Carnation Milk has been exposed to UV for a few seconds to increase the levels of vitamin D (Blake 1943). It asserted that "Irradiated Carnation Milk is pure and safe," "dependable and convenient," and "economical." Using a Centrifilmer, a type of equipment that produces a thin liquid film, UV was applied to egg white as a means of destroying bacteria, including *Salmonella* (Ijichi et al. 1964). At a feed rate of 100 ml/min, counts of *S. typhimurium* and *S. seftenberg* were reduced by 6 to 7 logs. Performance quality of egg

white was not affected adversely even by the maximum treatment, except for a slight coarsening of angel food cake texture. The development of commercial scale equipment was noted as requiring further study; however, this has yet to be done. The effect of UV on the inactivation of *Escherichia coli* O157:H7 in egg white was investigated (Ngadi et al. 2003). Egg white was placed in Petri dishes below a UV bulb. Agitation of samples was achieved using a vortex mixer. More than a 5-log reduction was obtained. The visual appearance of the treated egg white did not show any discoloration during 4 weeks of storage at ambient temperature.

A tubular UV processing system that is simple and straightforward was recently developed (Geveke 2005). It consisted of a single low-pressure mercury bulb surrounded by UV transparent tubing. The UV system inactivated both Gram-positive and Gram-negative bacteria at room temperature and at apple cider flow rates of up to 83 ml/min. The population of *E. coli* was reduced to below the limit of detection (equivalent to an inactivation of greater than 4.7 log). *Listeria innocua* was less sensitive to the UV treatment. Its population was reduced by 2.5 log. In both cases, the reductions followed first-order kinetics with respect to treatment time. The energy to UV process apple cider was 34 J/ml, which is comparable to that of thermal pasteurization (Kozempel et al. 1998).

UV pasteurization of egg white may be an attractive alternative to thermal processing and to other nonthermal processing. UV processing inactivates bacteria at temperatures well below the coagulation temperature range; it is accepted in the food industry, and its costs are similar to thermal processing. The main obstacle to commercializing UV processing of egg whites may be the lack of large-scale equipment. The objective of the present work was to substantially scale up the tubular UV processing system to a higher flow rate and to apply it to the inactivation of *E. coli* in liquid egg white.

Materials and Methods

The UV processing research was performed in a food pilot plant, so pathogenic bacteria could not be used. *E. coli* K12 (ATCC 23716) was maintained on tryptic soy agar (Remel, Lenexa, KS, USA) at 4 °C. Before inoculation of the product, the organism was cultured in tryptic soy broth (Remel) with shaking at 37 °C for 16–18 h. Pasteurized liquid egg white in cartons was obtained from a local supermarket. The egg white was inoculated from the stationary phase culture to give an approximately 6 to 7 log cfu/ml population. Processing began after a 2-h waiting period to allow the *E. coli* to become adapted to the basic environment. The pH of the egg white was 8.8. The pH of freshly laid eggs may be less than 8.0, and as the egg ages, the pH may rise to 9.0. Therefore, to determine the effect of pH on UV inactivation, in some cases, the pH of the egg white was adjusted to between 7.0 and 9.0 before adding the culture. Either 1.0 N hydrochloric acid or 1.0 N sodium hydroxide was used depending on the need. The populations of the untreated cells were negligibly affected by the pH adjustment (<0.1 log).

The UV process consisted of pumping egg white through UV transparent tubing that was coiled around germicidal UV bulbs. This concept was previously used to inactivate bacteria in apple cider (Geveke 2005). In that study, the UV processing rate was limited to 83 ml/min because of pressure drop restrictions. For the present study, a UV system with a higher flow rate that was closer to industrial scale was desired. The experimental equipment that was designed and assembled used four UV low-pressure mercury bulb units in series. Each unit contained a bi-pin base (model S130 120 LPF, Lithonia Lighting, Conyers, GA, USA) and a 30-W bulb (model G30T8, Buylighting.com, Burnsville, MN, USA) that generated 90% of its energy at a wavelength of 254 nm, which inactivates bacteria, molds, yeast, and viruses (Koller 1952). The cylindrical bulbs were 89.4 cm in length and 2.54 cm in diameter, and had a rated life of 8,000 h. Norton Chemfluor 367 UV transparent tubing (Cole-Parmer, Vernon Hills, IL, USA) with an internal diameter of 3.2 mm and a wall thickness of 1.6 mm was wrapped around the entire length of the UV bulb. The length of the tubing in contact with each bulb was 14 m. The tubing has a UV transmission at 254 nm of 89%. Self-fusing silicone rubber tape (McMaster-Carr, Robbinsville, NJ, USA) with a width of 2.5 cm and a thickness of 0.05 cm was wrapped around the plastic tubing to secure the tubing to the UV bulb and to ensure that no UV light escaped (Fig. 1).

The experimental system included a feed tank, pump, heat exchanger, tubular UV units, and a cooling coil. A progressing cavity pump (model ADBP 8.3, Allweiler, Radolfzell, Germany) pumped the egg white through a plate heat exchanger (model FT74-30-MkIII-33-34, Armfield, Jackson, NJ, USA) where the temperature was adjusted to 30, 40, or 50 °C. The feed rate was 330 ml/min and was the maximum that the Armfield equipment would allow based on pressure limitations. The egg white then flowed through the UV transparent Chemfluor tubing. One, two, three, or four UV bulbs were used in series, depending on the experiment. At the flow rate of 330 ml/min, the UV treatment time was 20 s per bulb. Hence, the treatment times ranged from 20 to 80 s.

The temperatures of the egg white entering and exiting the tubular UV bulb units were measured with 3.2 mm diameter chrome-constantan thermocouples (Omega Engineering, Inc., Stamford, CT, USA). The UV bulbs gener-

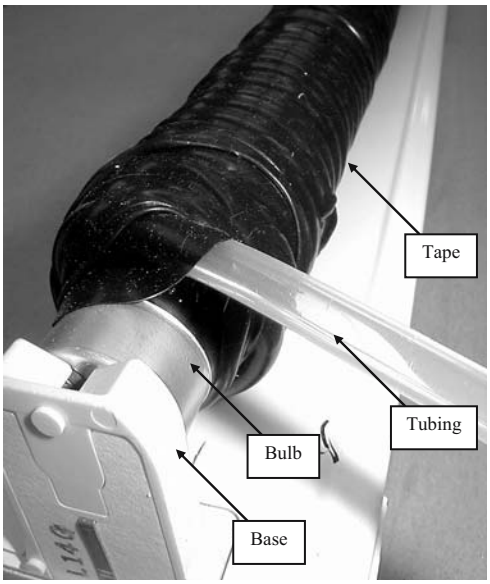

Fig. 1 UV bulb unit consisting of bi-pin base, 30 W germicidal UV bulb, UV transparent tubing, and silicone rubber tape

ated a slight amount of heat. At inlet temperatures of 30 and 40 °C, the temperatures of the egg white increased by an average of 0.5 and 0.1 °C per bulb, respectively. At an inlet temperature of 50 °C, the heat loss to the surroundings was greater than the heat produced by the UV bulbs and the temperature of the egg white decreased by an average of 0.7 °C per bulb. The temperatures were continuously logged to a data acquisition system (Dasytec USA, Amherst, NH, Dasylab version 7). After UV treatment, the egg white was cooled to <25 °C in approximately 2 s using a stainless-steel cooling coil submerged in a water bath.

In some cases, the effect of exposure to longer treatment times was desired and the egg white was reprocessed. Product from the outlet of the cooler was collected in a carboy and was processed through the system a second time.

Controls were performed to determine the effect of temperature alone. The UV bulbs were turned off, and the egg white was heated to 50 °C using the heat exchanger and then cooled using the cooling coil.

The experimental design is presented in Table 1. The UV treatment time ranged from 0 to 160 s, the temperature ranged from 30 to 50 °C, and the pH of the egg white ranged from 7.0 to 9.0.

Product samples were collected in polypropylene tubes and placed on ice in a dark location to prevent photoreactivation (Clarke and Berman 1983). Appropriate dilutions of the product samples were made in Butterfield's phosphate buffer (Hardy Diagnostics, Santa Maria, CA, USA) with a minimum 1 ml transfer. Duplicate samples were then pour plated with tryptic soy agar (Remel) and the plates incubated at 37 °C for 24 h. Plates with 30–300 colonies were enumerated using a manual colony counter (Bantex, Burlingame, CA, USA, model Colony Counter 920).

Each UV experiment was performed in duplicate. Results were expressed as the means of these values ± the standard deviations calculated using Microsoft Excel statistical analysis algorithms.

The energy to process liquid egg whites using the UV system in this study was calculated for the case where the egg white received the maximum treatment time (i.e., where the egg white was processed twice using four UV bulbs). Each bulb is rated at 30 W; thus, eight bulbs require 240 W of power. In continuous processes, a common method of comparing efficiencies is to calculate the energy per flow rate. Based on the flow rate and the UV bulb wattage, the energy applied was 44 J/ml as determined by the following equation:

$$\text{Energy density} = \frac{\text{Power}}{\text{Flow rate}} = \frac{240\,\text{W}}{330\,^{\text{ml}}/_{\text{min}}} \times \frac{1\,^{\text{J}}/_{\text{s}}}{1\,\text{W}} \times \frac{60\,\text{s}}{\text{min}} = 44\,^{\text{J}}/_{\text{ml}}$$

Results and Discussion

In most antimicrobial processes, inactivation improves with increasing temperature. This is true for thermal as well as the majority of nonthermal processes. To investigate the

Table 1 UV conditions used in this study

Temperature, °C	pH	Time, s	UV bulbs
30	8.8	80	On
40	8.8	80	On
50	8.8	80	On
50	8.8	80	Off
50	7.0	80	On
50	7.0	80	Off
50	7.5	80	On
50	8.0	80	On
50	8.5	80	On
50	9.0	80	On
50	9.0	80	Off
50	8.8	20	On
50	8.8	40	On
50	8.8	60	On
50	8.8	100	On
50	8.8	120	On
50	8.8	140	On
50	8.8	160	On
50	8.8	160	Off

Controls (without UV) were performed with the bulbs turned off.

effect of temperature on UV inactivation of *E. coli* in liquid egg white, experiments were performed at 30, 40, and 50 °C at a constant treatment time of 80 s. The population of *E. coli* in egg white was reduced by 1.63±0.10 log at a temperature of 30 °C (Fig. 2). When the temperature was raised to 40 °C, the population was reduced by 2.32± 0.07 log. The inactivation at 50 °C was 2.48±0.41 log. To determine the effect of temperature alone, egg white was heated to 50 °C using the heat exchanger and pumped through the UV system with the bulbs turned off. The thermal inactivation at 50 °C was negligible at 0.13± 0.03 log. Thus, the vast majority of the 2.48 log UV inactivation at 50 °C was due to nonthermal effects.

UV inactivation improved as the temperature increased from 30 to 50 °C. This phenomena has been observed before in other nonthermal processes such as high-pressure processing (Alpas et al. 2000; Stewart et al. 2000), pulsed electric fields processing (Jeantet et al. 1999; Zhang et al. 1995), and radio frequency electric fields processing (Geveke and Brunkhorst 2003, 2004a). Enhanced inactivation at temperatures above 30 °C may be due to the phase transition of the phospholipid molecules, within the cell membrane, from gel to liquid-crystalline (Jayaram et al. 1992). Another reason for the improved inactivation at higher temperatures may be due to the nature of low-pressure UV bulbs. The production of 254 nm UV irradiation increases with respect to ambient temperature up to a maximum level (Koller 1952). Then, it drops off with increasing temperature. For a 2.54-cm diameter bulb, the optimum output occurs at a temperature of 40 °C (Forsythe et al. 1941).

From an industrial standpoint, there would be minimal additional expense to process liquid egg white with UV at 40 to 50 °C rather than at lower temperatures. This is because the bulk of the heat required to raise the feed

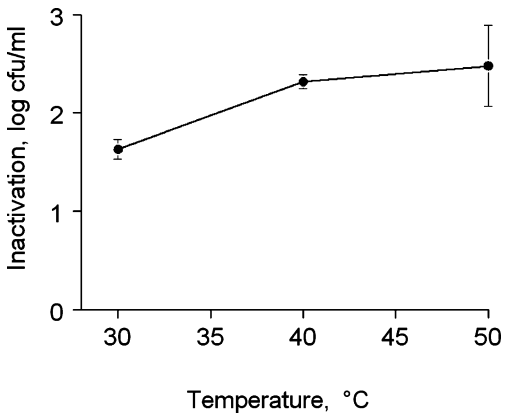

Fig. 2 Effect of UV processing temperature on the inactivation of *E. coli* in liquid egg white at a treatment time of 80 s and a pH of 8.8. Means of two replicate experiments. *Error bars* indicate standard deviations

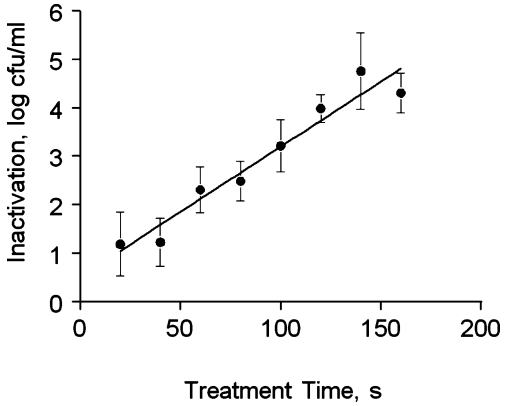

Fig. 3 Effect of UV treatment time on the inactivation of *E. coli* in liquid egg white at a temperature of 50 °C and a pH of 8.8. Means of two replicate experiments. *Error bars* indicate standard deviations. Control (without UV) at 160 s resulted in <0.1 log inactivation

temperature could be recovered from the product using a heat exchanger.

A series of experiments were performed to determine the effect of treatment time on inactivation at a constant temperature of 50 °C. Using one to four UV bulbs in series, the treatment times investigated were 20, 40, 60, and 80 s. To obtain longer treatment times and to achieve greater microbial inactivation, some of the product was collected and reprocessed to provide total treatment times of 100, 120, 140, and 160 s. The population of *E. coli* in egg white was reduced by 1.18±0.66 log at a treatment time of 20 s (Fig. 3). When the treatment time was increased to 160 s, the population was reduced by 4.30± 0.41 log. The data follow first-order kinetics with a multiple regression correlation coefficient (r^2) of 0.94. First-order kinetics has also been obtained with PEF and thermal treatments (Espachs-Barroso et al. 2003). The calculated *D* value is 37 s.

As an egg ages, its pH increases by the loss of CO_2 through the shell. The pH of egg white from a newly laid egg is approximately 8.0 and increases to 8.6 and 9.1 after being stored for 24 and 72 h, respectively (Heath 1977). As transportation from the farm to the breaking plant and processing are becoming more efficient within the egg industry, the pH of liquid egg white has trended downward over the past few decades. To determine whether UV inactivation of *E. coli* is affected by the pH of the egg white, experiments were performed at pH values from 7 to 9. At 50 °C, a treatment time of 80 s, and a pH of 7, the population of *E. coli* in egg white was reduced by 2.80± 0.12 log (Fig. 4). When the pH was increased to 9, the population was reduced by 1.68±0.16 log. Therefore, UV inactivation of *E. coli* in egg white is greater at neutral than at higher pH values. The reason for this is unclear but may be related to the fact that most egg white proteins attain

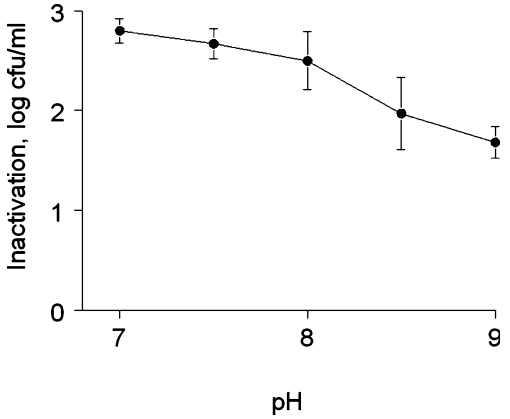

Fig. 4 Effect of pH on the UV inactivation of *E. coli* in liquid egg white at a temperature of 50 °C and a treatment time of 80 s. Means of two replicate experiments. *Error bars* indicate standard deviations. Controls (without UV) at pH values ranging from 7 to 9 resulted in <0.1 log inactivation

maximum stability at near-neutral pH (Stadelman and Cotterill 1995). The opposite effect occurs in thermal inactivation (Palumbo et al. 1996). That is, as the pH of egg white decreases, thermal inactivation becomes more difficult, and the recommended pasteurization temperature increases (Stadelman and Cotterill 1995).

The energy to process liquid egg whites is important because the liquid egg industry, like many other food industries, operates on tight margins. The processing energy for the case where the egg white received the maximum treatment time was 44 J/ml. This value is in relatively fair agreement with that obtained in a previous study on UV inactivation of bacteria in apple cider using a smaller scale UV system (Geveke 2005). In that investigation, the calculated energy was 34 J/ml. The UV transmittances of apple juice and liquid egg are similar (Ngadi et al. 2003). Therefore, the higher required energy obtained in the present study may be due to the effect of scaling up the process. The small-scale UV system contained tubing with an ID of 1.6 mm and a wall thickness of 0.8 mm; whereas the UV system used in the present study had tubing with an ID of 3.2 mm and a wall thickness of 1.6 mm.

For comparison, the energy for conventional thermal pasteurization, with heat regeneration or recovery, is approximately 35 J/ml (Kozempel et al. 1998). Thus, the energies of UV processing and thermal processing are similar. For additional comparison, the energy for other nonthermal processes, pulsed electric fields, and radio frequency electric fields is estimated to be in the range of 100–400 J/ml (Barsotti and Cheftel 1999; Geveke and Brunkhorst 2004a, b; Geveke et al. 2006; Schoenbach et al. 2002).

Ijichi et al. (1964) observed an off odor induced by UV processing of egg white. The egg whites were evaluated in angel food cakes. It was concluded that, at intermediate UV intensities, the use of UV processed egg whites in flavored angel food cakes was possible. In the present study, an off odor in raw UV-processed egg white was also observed. In the future, the effect of UV processing on the organoleptic properties of egg white will be examined. Potential evaluations could include determining the volume, coarseness, and sensory characteristics (by a trained taste panel) of unflavored angel food cakes prepared from the whites, meringue foaming power, and foam stability. In addition, the extension of the UV process to whole liquid egg and the further scaling up of the process will be investigated. Furthermore, studies should be performed to determine the extent of sublethal injury and the possibility of forming harmful substances. Finally, a UV system will be designed and assembled for use with pathogens such as *S. enteritidis* in a Biosafety Level 2 laboratory.

Conclusions

A UV system was developed and assembled that inactivated *E. coli* in liquid egg white. It was based on a small-scale system that previously had been used to inactivate bacteria in apple cider. The UV system used in the present study consisted of four low-pressure mercury bulbs each surrounded by UV transparent tubing. The population of *E. coli* in egg white was reduced by 2.5 log after being exposed to UV at 50 °C for 80 s. When the UV treatment time was reduced to 0 s (i.e., with the UV bulbs turned off), and the temperature was maintained at 50 °C, the reduction was 0.1 log. Therefore, the majority of the UV inactivation at 50 °C is due to nonthermal effects. Reducing the pH of the egg white from 9 to 7 increased the inactivation of *E. coli* from 1.7 to 2.8 log, while reducing the UV processing temperature from 50 to 30 °C decreased inactivation from 2.5 to 1.6 log. The effect of UV treatment time on inactivation was successfully correlated using a first-order model. For a treatment time of 160 s, the population was reduced by 4.3 log. The calculated *D* value was 37 s. The electrical energy necessary to obtain a 4.3-log reduction of *E. coli* was calculated as 44 J/ml, which is approximately the same as the energy required for thermal pasteurization and is substantially less than that for other nonthermal processes. Thus, the UV process, developed to inactivate bacteria in apple cider, has been effectively scaled up, and its application has been extended to liquid egg white.

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